Proc. Roy. Soc. Victoria, 25 (N.S.), Pt. II., 1912.

Art. XVII.—*On the Cross Inoculation of the Root Tubercle Bacteria upon the Native and the Cultivated Leguminosæ.*

Preliminary Communication

BY

A. J. EWART, D.Sc., Ph.D., F.L.S.

(Professor of Botany and Plant Physiology in the University of Melbourne),

AND

NORMAN THOMSON, B.Ag.Sc.

(Government Research Bursar, Melbourne University).

(With Plate XIV).

Read 8th August, 1912.

Introduction.

If the roots of any leguminous plant are examined, there will always be found certain little round growths attached to them; these are known as nodules or tubercles, and contain countless numbers of bacteria. It is calculated that one such tubercle of an average size (that of a match-head) would contain from 100 to 1000 million bacteria.

It has been found that the growth of any leguminous crop sown in certain new sandy or heathy ground, will receive a decided impetus if soil from a field in which this same crop has been recently grown is scattered over it, either before or after sowing. This is due to the introduction of this bacterium *Bacillus radicicola* (Beyerinck) present in the soil, which causes the formation of the nodules upon the roots, entry into which is gained through the root hairs. In these nodules the bacteria exercise their power of fixing free nitrogen from the air, and passing it on to host plants in forms it can utilise as nitrogenous food supplies.

The Problem.

There is a difference of opinion as to whether inoculation can be carried out on any one plant by the bacteria from the tubercles of that plant only, or from any other plant of the same genus, or

indeed, from any cultivated legume at all. The point of special local interest was to determine whether the bacteria from the root nodules of native leguminosae were able to directly infect the leguminosae (lucerne, peas, clover, beans etc.), not native to Victoria, but commonly cultivated.

In the many papers published upon this subject, there has been mention of little work in connection with legumes other than those cultivated, and none which could be regarded as affording a complete and final answer to this question. Nobbe and Hiltner[1] had at different times been able to inoculate the bean plant with bacteria present in the nodules from peas, and Dr. Moore[2] had also successfully carried out cross inoculation on some cultivated legumes.

Its Bearing on Economic Agriculture.—However, since nothing of a definite nature had been done with the native legumes of any country, it seemed worth while carrying out experiments with the better known and more widely spread legumes of this State, and to settle, if possible, the question whether the bacteria from various wild and cultivated plants were all alike as regards their power of cross infection, or whether biological races of the root nodule bacteria existed, each capable of infecting either one plant only or a few plants of similar physiological character. In particular, the question whether the same common and widely spread bacteria, which live upon and enrich such native plants, as for instance *Acacia*, were capable of living in symbiosis with the commoner cultivated members of this order, had an important bearing on agriculture, particularly in regard to the practice to be adopted in opening new ground to cultivation. For instance, if all the root nodule bacteria are capable of direct cross infection, then virgin country, whose flora comprise such plants as *Acacia*, *Swainsona*, *Platylobium*, etc., will, on being cleared, and given over to the cultivation of peas, lucerne, clover, or any other such crops, possess in the soil their necessary adjuncts, the nodule bacteria, which on entering into symbiotic union would cause a more luxurious growth than could otherwise be possible, and without the use of heavy dressings of nitrogenous manures. On the other hand, if the bacteria from native legumes were unable to infect cultivated ones, they must be introduced into the soil in some way, preferably by the means already mentioned.

1 Nobbe. Versuchsst. 1894. Bd. xlv., p. 155.
Nobbe and Hiltner. Centbl. Bakt. u. Par. 2, Abt. 6 (1900), No. 14, pp. 449-457, pl. 1.

2 Dr. G. T. Moore. Inoculation of Soil for Leguminosae. United States Bur. Pl. Ind., 17, 1905.

Method of Attacking the Problem.—Eight of the more important members of the Leguminosae were taken, viz. :—

1. Broad Bean ... ... Vicia Faba.
2. Kidney Bean ... ... Phaseolus vulgaris.
3. Field Pea ... ... Pisum sativum.
4. Soy Bean ... ... Glycine hispida.
5. Lucerne ... ... Medicago sativa.
6. Black Medick ... ... Medicago lupulina.
7. Red Clover ... ... Trifolium pratense.
8. Hairy Vetch ... ... Vicia villosa.

For cross inoculation purposes, the following plants were chosen :—

1. Acacia mollissima ... ... Mimosae.
2. Platylobium obtusangulum ...
3. Swainsona galegifolia ... ...
4. Aotus villosa ... ... } Papilionaceae.
5. Bossiaea cinerea ... ...

Arrangement of Series of Pots.—According to Hiltner and Stormer[1], if pot experiments were to be carried out, inoculation by nodule contents was the best way; but for field work, inoculation by pure cultures of the bacteria yielded the best results. Since it was only possible to keep everything sterile in pot experiments, the eight different kinds of seeds were planted in pots, and each series was inoculated by the bacteria from a different native legume, thus giving five series, a sixth being planted uninoculated, and comprising a control, a total of 48 pot plants being used for each experiment.

As the risk of contamination and outside infection must be completely overcome, everything before use was thoroughly sterilised.

Method of Sterilisation and Precautions.—The pots before sowing were placed in the steamer and kept at boiling point for 2 hours, 2 days later being resubjected to the same treatment; this would effectively get rid of any foreign root tubercle bacteria that might be present. The seeds were well washed for a few minutes in a 2 per cent. solution of formaldehyde, and then several times in boiled water. This treatment seemed effective enough, yet Harrison and Barlow[2] (Canadian investigators) say that the various methods of sterilisation are of no avail in removing the living bacteria from the living seeds; and, in fact, in two cases with clover plants nodules appeared whose origin was difficult to explain except on the

1 L. Hiltner and K. Stormer. Berlin, Arb. biol. Abth. Gesundhtsamt, 3, 1903 (151-307, 445-545).

2 F. C. Harrison and B. Barlow. The nodule organism of the Leguminosae, its isolation and cultivation, identification and commercial application. (Pseudomonas radicicola). Canada, 1907.

assumption that they might have been caused by bacteria remaining on the seed after ineffective sterilisation. Boiled water was used in all cases, and all jars and beakers were placed in boiling water before use to ensure perfect sterilisation and pure inoculation for each series.

Manner of Infection and Sowing.—To infect the seeds, an infusion in water was made of the contents of the nodules from each of the chosen legumes. The nodules were washed thoroughly in sterilised water many times, no disinfecting agent being used for fear of killing the bacteria; they were then crushed up in water, and the seeds before sowing were soaked in this bacterial infusion, each series, of course, in its own inoculating material. After

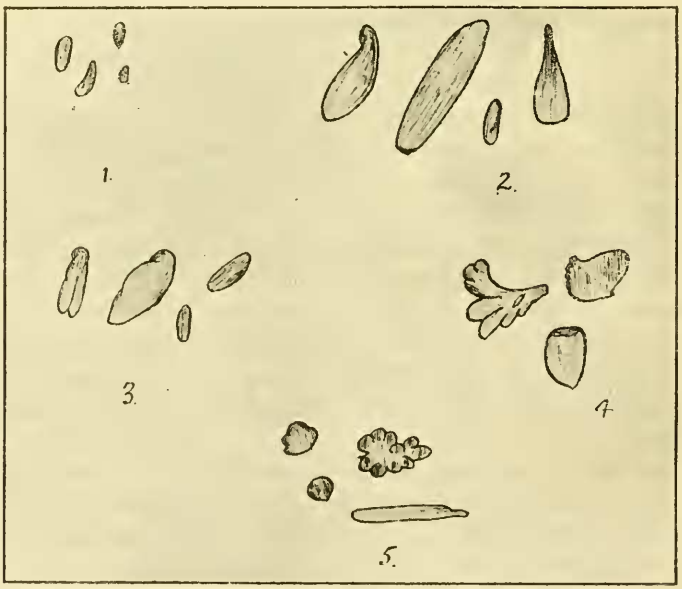


FIG. I.—LIFE-SIZE DRAWINGS OF NODULES.

1. Platylobium nodules ... 2-4 mm. x 1-2 mm. Colour dark brown, white towards one end. Oval shape.
2. Aotus ,, ... 6-20 mm. x 2-5 mm. Light brown colour. Very elongated, though regular shape.
3. Bossiaea ,, ... 5-12 mm. x 2-4 mm. Creamy brown, and regular oval shape.
4. Acacia ,, ... 6-10 mm. x 4-5 mm. Light brown one end, white the other. Irregular rounded and oval.
5. Swainsona ,, ... 3-20 mm. x 2-3 mm. Brown and white, and very irregular shape, somewhat elongate. Many grouped in bunches.

sowing, each infusion was diluted with the boiled water, and portion of it used to water the pots, the remainder being further diluted and used for three subsequent waterings spread over four or five days, so that in this way, the bacteria were brought into intimate contact with the seeds and young roots.

Possible results of Cross-Inoculation.—The nodules from the various plants showed great differences in size, form and colour, each kind being easy of recognition. If nodule growth on the plants followed inoculation, then it seemed feasible that the size and shape of the mother nodule should be reproduced in the nodules formed upon the roots of the inoculated host plant.

By microscopic examination it was noticed that there were distinct differences in shape and size between the bacteria from the different native legumes, and that the root nodule bacteria from native plants, were much larger than those examined from the nodules of clover and lucerne.

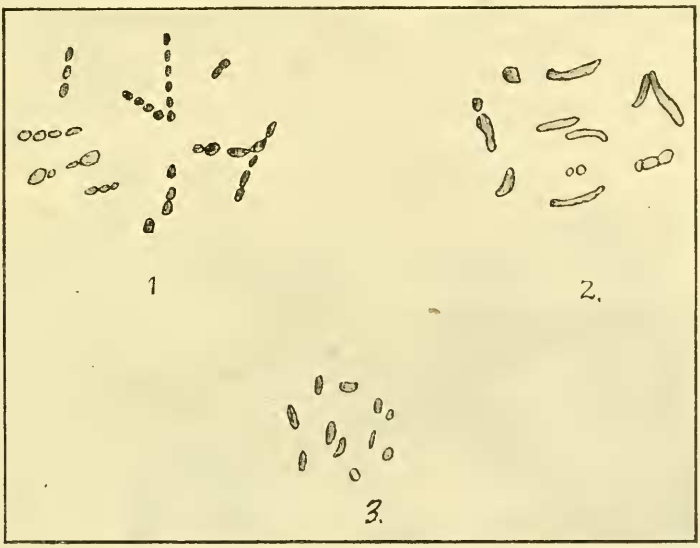


FIG. II.—DRAWINGS OF BACTERIA FROM NODULES OF THREE DIFFERENT NATIVE LEGUMINOSAE (× 690).

1. *Platylobium* nodule bacteria. Congregate in chains, and are small round bacteria.
2. *Acacia* nodule bacteria. Curved rods of varying length, rounded ends. Longer than *Platylobium*
3. *Swainsona* nodule bacteria. Bacteria found singly; short and stumpy, but of regular size.

If the cross-inoculation were successful, it would be interesting to see whether the nodules produced upon the infected plant were not only the same externally, i.e., in size and colour, as those on the infecting plant, but also internally; whether the histology and anatomy were the same, the cells, the cork tissue, the vascular bundles, the bacteria containing cells, etc., were identical in each. If this were so, one would expect the bacteria in the nodules produced upon the roots of clover inoculated with acacia nodule bacteria, the clover bacteria being smaller than the latter, to be of a similar size and shape to the acacia bacteria and of similar physiological and biological characters.

Actual results in tabular form.—The experiments were tried twice, extending over a period of seven months, November, 1911, to June, 1912. After sowing, the plants were allowed varying periods of growth before examination, from seven weeks in the case of most to four months in that of clover and lucerne in the first experiment.

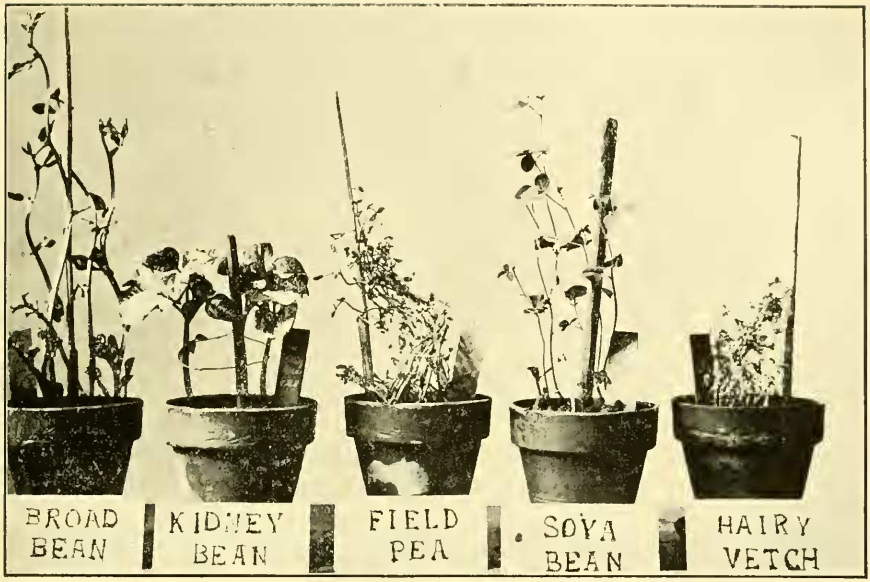
Photographs were taken of the five most forward plants in two series, that uninoculated and the one inoculated with root nodule bacteria from *Platylobium* six weeks after sowing. Although the results show that no nodules were present on the roots of the inoculated series of plants, yet they showed a more decided growth, and were larger and stronger. Whether this was due to the root nodule bacteria continuing to live in the soil and fix nitrogen outside the plants they were unable to infect, is an open question. (See Plate XIV.)

The results may be shown in the following table:—

ROOT TUBERCLES DEVELOPED AFTER INOCULATION WITH BACTERIA TAKEN FROM NATIVE LEGUMES, AS UNDER.

Cultivated Legumes.	Acacia.		Platylobium.		Swainsona.		Aotus.	Bossiaea.	Uninoculated.	
	1st.	2nd.	1st.	2nd.	1st.	2nd.	2nd.	2nd.	1st.	2nd.
1. Broad Bean	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
2. Kidney Bean	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
3. Pea	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
4. Soy Bean	nil	nil	nil	died	nil	died	died	died	nil	died
5. Lucerne	nil	nil	died	nil	died	nil	nil	nil	nil	nil
6. Black Medick	nil	nil	nil	nil	nil	died	nil	nil	died	nil
7. Clover	2	nil	nil	nil	nil	nil	nil	nil	several	nil
8. Vetch	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil

The above table shows that of 80 different lots of plants that were sown, on only two of these, clovers, were nodules found. The uninoculated clover possessed many nodules, and microscopic examination showed these to be packed with bacteria of the usual kind. It is possible that in this case and in the clover infected by


BROAD BEAN
KIDNEY BEAN
FIELD PEA
SOYA BEAN
HAIRY VETCH


1
3
5
BROAD BEAN
KIDNEY BEAN
FIELD PEA
SOYA BEAN
HAIRY VETCH